

Iron Formulation Affects In Vitro Cold Storage of Hops

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Abstract

Improving the duration of cold storage of in vitro germplasm collections is important to safeguarding plant diversity available for plant breeding. In this study, we characterized the response of 12 diverse *Humulus lupulus* L. genotypes to the iron formulation used in the growth medium during storage. Treatments were standard MS iron alone (EDTA chelated) or with 100 or 200 mg/L sequestrene 138 iron (EDDTA chelated). In standard storage conditions, the average length of storage for hops on MS medium is 14.1 ± 3.5 months. Evaluation of 12 genotypes after 3, 6 and 9 months indicated that the plantlets grown with standard MS iron had generally higher condition ratings than those on sequestrene iron. Plants on either concentration of sequestrene iron declined at all rating periods and by 9 months all were near death. When all 12 genotypes were considered as a group, the growth-condition ratings for plantlets stored on MS iron were not significantly different from those on either sequestrene iron concentration due to the large amount of variation among the genotypes. Plants stored for 9 months on 1X sequestrene iron declined from mean ratings of 5 to ratings of 1.2 ± 1.1 and those on 2X declined to 0.8 ± 0.9 while plants on MS were rated 2.7 ± 1.5 . Significant differences among treatments were noted for several genotypes. These results indicate that 4°C storage of in vitro hops germplasm should be on medium with the standard MS iron formulation.

INTRODUCTION

The USDA-ARS National Clonal Germplasm Repository (NCGR) stores the global diversity of *Humulus* (hops) for the US Plant Germplasm System. The primary collection of hops is held as trellised plants in a field genebank. To insure the safety of the collection, a subset of the field collection was established as virus-free in vitro cultures stored at 4°C and as potted plants grown in screened houses. Breeders and government facilities use collections of valuable plant germplasm to develop cultivars with improved crop yield and disease and insect resistance characteristics. Exotic germplasm requires safe storage. Obscure genes from these plants may solve new disease, insect, or environmental or crop production problems (Westwood, 1989). *Humulus* (hops) germplasm is preserved as clonal field-grown or potted plant. These plants can be readily cultured in vitro for back-up collections (Reed et al., 2003). Some clonal crops are kept in slow-growth storage as in vitro cultures (Ashmore, 1997; Engelmann, 1991; Withers, 1991; Withers et al., 1990). Nine genera at NCGR are held as in vitro cold-stored cultures at NCGR (Reed, 1999 b). Length of storage at 4°C is dependent on many factors (Ashmore, 1997). Cold acclimation (Reed, 1993), nitrogen concentrations (Moriguchi and Yamaki, 1989) the growth regulator concentrations in the medium (Reed, 2002), container used (Reed, 1991; 1992) and photoperiod (Reed, 2002) are all important factors in the longevity of stored plants.

Humulus culture medium is often an MS-salts based medium (Murashige and Skoog, 1962). Sequestrene iron is used in plant growth media for various purposes (Tsao and Reed, 2002). We recently determined that culture medium with sequestrene iron greatly improved leaf color and growth of many species and cultivars of hops. (Reed, unpublished). This study was designed to determine if the improved vigor of growth-

room plantlets would be maintained during cold storage on medium with sequestrene iron and if longer mean storage duration would be possible for most accessions.

MATERIALS AND METHODS

Plant Materials

Twelve *Humulus lupulus* L. accessions were used in this study: Alpha Aroma, Cascade, Colorado 1-3, Hallertauer Mittlefrüher, Hallertauer Tradition, Hersbrucker-8, Mt. Hood, Pacific Gem, Perle, Spalter Select, USDA 21119, and Vojvodina.

Culture Conditions

In vitro cultures were originally initiated from 0.3 to 0.5 mm meristems of heat-treated shoots from clonally propagated hops plants (Adams, 1975). Plantlets were grown on NCGR-HUM medium [MS (1962) salts and vitamins with 2% glucose, 4.4 μM N⁶ benzyladenine, at pH 5.0 and solidified with 0.3% agar and 0.125% Gelrite] before use in this study (Reed et al., 2003). Shoots were multiplied on 40 ml of medium in Magenta GA-7 vessels at 25°C under a 16-h photoperiod (40 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

Cold Storage of In Vitro Cultures

Storage followed the technique developed for other genera, but NCGR-HUM medium was used. Plantlets with 2 nodes (2 to 3 cm height) were transferred to 5-chambered semi-permeable tissue-culture bags (StarPac, Garner Enterprises, Willis, Tex) with 10 ml medium per chamber 3 wk after the last regular subculture. Storage medium was NCGR-HUM medium without growth regulators with one of the treatments described below and was gelled with 0.35% agar and 0.145% Gelrite™. Ten plantlets of each accession were prepared for each treatment with each plantlet in an individual section (15 x 150 mm) of a five-section bag.

Cold Acclimation

Plants in bags were grown for 1 wk under growth-room conditions then cold acclimated for 1 wk in a growth chamber with temperature/photoperiod settings of -1°C 16-h dark/ 22°C 8-h light (10 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) as the standard treatment (Reed, 1993).

Experimental Treatments

Iron treatments were standard MS medium (iron alone, EDTA chelated) or addition of 100 or 200 mg/L sequestrene iron 138 (EDDTA chelated). Storage was at 4°C with a 12-h photoperiod and very low light (3 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Twelve accessions with six bags each were analyzed.

Visual Evaluation

Hop plantlets were evaluated at planting and at 3 and 6 months. Each plantlet was rated on a 0 to 5 scale. Ratings were: 5, dark green leaves and stems, no etiolation, base green; 4, green leaves and stems, little etiolation; 3, shoot tips and upper leaves green, etiolation present, base green; 2, shoot tip green, leaves and stems mostly brown, base may be brown; 1, plantlet mostly brown, only extreme shoot tip green, much of base dark brown; 0, all of plantlet brown, no visible green on shoot tip. Plantlets were repropagated when ratings were ≤ 2 (Reed et al., 1998).

RESULTS AND DISCUSSION

When all 12 genotypes were considered as a group, the growth-condition ratings for plantlets stored on MS iron were not significantly different from those on either sequestrene iron concentration (Fig. 1). The differences were not significant due to the large amount of variation among the genotypes, but the plants stored for 9 mo on 1X sequestrene iron declined to mean ratings of 1.2 ± 1.1 and 2X declined to 0.8 ± 0.9 while those on MS were rated 2.67 ± 1.5 . Significant differences among treatments were noted

for several genotypes (Fig. 2). Cascade was the only genotype that responded poorly to growth on all three iron formulations, but this response was quite different from our earlier studies where this genotype retained viability for over 12 months on medium with MS iron. AlphaAroma, Hersbrucker-8, Perle and USDA 2119 all stored significantly better with MS iron compared to sequestrene iron at 3, 6 and 9 months. In all cases most of the plantlets grown on MS iron were in equal or better condition than those on either sequestrene iron concentration. The goal of cold storage is to keep plantlets stored as long as possible while still retaining viability. In 9 of the 12 genotypes the mean ratings for 9-month stored plantlets on MS iron remained well above the “2” rating given to plants in need of growth room re-propagation. Only 7 of the 12 stored on sequestrene iron were rated >2 at 6 months and all were <2 by 9 months. Given the trend shown in this data, it is likely that plantlets of most accessions stored on sequestrene iron will be dead at 12 months while those on MS will remain in storage.

Reed et al., (2003) found that *Humulus* plantlets (on MS iron) remained viable in cold storage for an average of 14.1 ± 3.5 months. Individual accessions varied in the length of viability during storage from 6 to 26 months and cultivar storage was similar (14.6 ± 3.4) to that of the wild accessions (12.6 ± 3.2). Variation also occurred between storage cycles due to many factors. When compared to data from the 2003 study, the 9 genotypes also in this study remained viable in storage, with ratings >2, from 9 to 21 months.

Many factors must be assessed when determining the best conditions for storing in vitro cultures (Ashmore, 1997; Moriguchi and Yamaki, 1989; Reed, 1999a; Reed and Chang, 1997; Reed et al., 1998). Optimizing the medium for many individual factors could require long periods of study for each genotype. The main goal is finding a combination of factors that will provide a reasonable length of storage (>12 months) for all accessions. For hops, we found that regular culture medium with sequestrene iron greatly improved leaf color and growth of many accessions in the growth room (Reed, unpublished data). In sharp contrast to the improved growth of *Humulus* accessions at 25°C, this study shows that adding sequestrene iron to the storage medium generally resulted in much shorter storage than the standard MS iron formulation. Iron is an essential mineral for growth and development of plants; however it is also involved in free-radical mediated oxidative stress (Benson et al., 1995). Secondary oxidative stress manifests as browning, necrosis, and death of tissues exposed to low temperatures (Benson, 1990). The improved iron availability that resulted in superior growth of cultures at 25°C was likely also the cause of more rapid decline of the plants in 4°C storage. Additional iron availability may be detrimental to growth of plant tissues at low temperatures due to the pro-oxidant properties of iron.

These results indicate that 4°C storage of in vitro hops germplasm should be on medium without supplemental sequestrene and include only the standard MS iron formulation. Additional studies are needed to determine if removing all iron from the medium would further improve culture storage.

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Figures

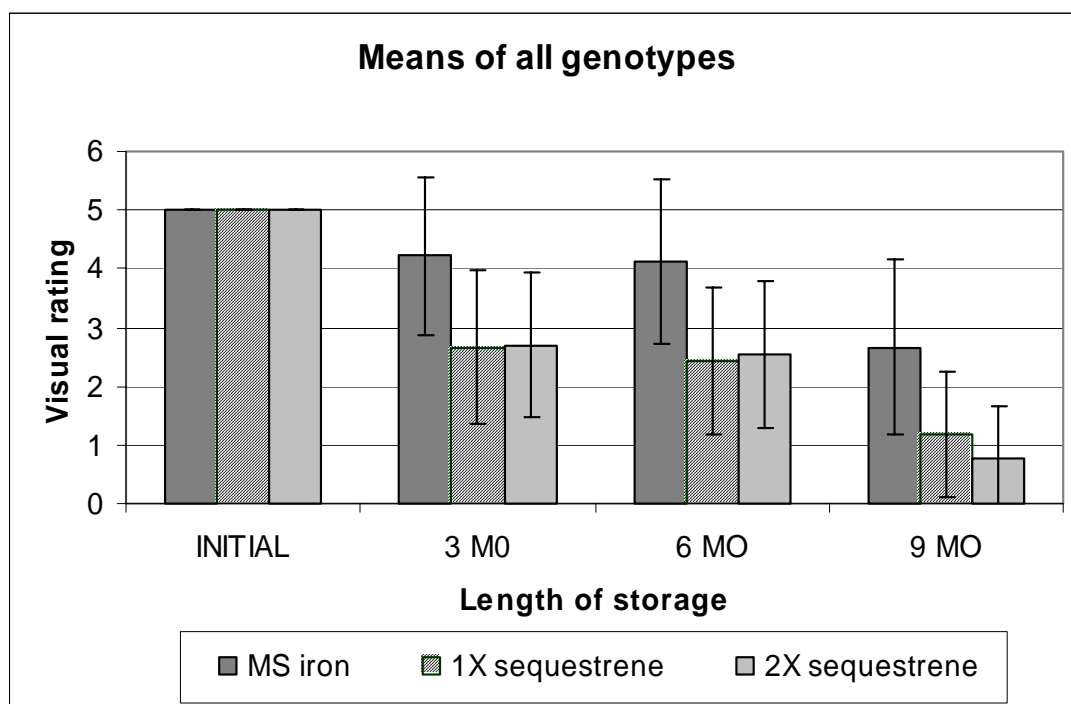


Fig. 1. Overall mean storage condition ratings \pm standard deviation of 12 *Humulus* genotypes after 3, 6, and 9 months of 4°C cold storage on NCGR-HUM medium with EDTA chelated iron (■) (MS formulation) alone or with 100 mg (◇) or 200 mg (▲) of EDDTA chelated iron (sequestrene 138) added per L.

Fig. 2. Mean storage condition ratings \pm standard deviation of 12 *Humulus* genotypes after 3, 6, and 9 months of 4°C cold storage on NCGR-HUM medium with EDTA chelated iron (■) (MS formulation) alone or with 100 mg (◇) or 200 mg (▲) of EDDTA chelated iron (sequestrene 138) added per L. (see next page).

